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Full Length Research Paper

Antioxidant activity and separation of phenolic compounds of *Origanum glandulosum* from north Algeria by high performance liquid chromatography (HPLC)

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Origanum glandulosum **is an endemic plant of North African area (Algeria and Tunisia), used against infections like whooping cough and bronchitis. In this study, its antioxidant activity is evaluated. The results showed that the scavenging effect against DPPH is equal to 6730 mg/ml (IC_{50}). However, the scavenger effect of H_2O_2 estimated at 60.78% is very strong compared to the effects of BHA, BHT and α-tocopherol estimated respectively at 19, 25 and 23%. The scavenger effect of H_2O_2 is very strong compared to the scavenging of DPPH. A high scavenging effect is generally due to high content of phenolic compounds, phenolic acids and flavonoids. The results may suggest that *O. glandulosum* extract possesses compounds with antioxidant activity; these substances can play an important role in the prevention of free radical induced diseases.**

Key words: Phenolic compounds, *Origanum glandulosum,* HPLC, antioxidant, flavonoids.

INTRODUCTION

Origanum glandulosum is an endemic widely distributed taxon of the North African region (Algeria and Tunisia). It is considered as brushwood (Quezel and Santa, 1963; Leclerc, 1990; Bendahou et al., 2008). Several studies have shown that *O. glandulosum* taxon is rich in essential oils, phenolic compounds such as flavonoids and phenolic acids (Benzanger-beauquesne et al., 1980, 1990; Nakiboglu et al., 2007). In Algeria, this plant is used against infections like whooping cough, cough, fever and bronchitis (Bendahou et al., 2008).

The purpose of this study was the estimation of the total phenolic content of *O. glandulosum* using the Classical Folin-Ciocalteu reagent, the determination of their antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH assay), the H_2O_2 scavenging effects and the analysis of the chemical composition of methanolic extract by HPLC chromatography, to estimate the number of major components of this plant. The choice of this investigation is based on two criteria; firstly, there are few studies in this domain in Algeria and the second criterion is the traditional utilization of *O. glandulosum.*

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Abbreviations: BHA, Butyl hydroxyanisol; **BHT,** butyl hydroxyltoluene; **DPPH**, 1,1 diphenyl-2-picryl hydroxyl.

MATERIALS AND METHODS

Plant materials

Samples of aerial parts (leaves, twigs and flowers) of individual plants were collected in the region of Boukhlifa (Béjaia) (36°41' 19°, 39' N; 5°06' 26°, 56' S; alt.1.00 Km) during June 2007. Identification of plants was made by the botanical laboratory of Béjaia University.

Crude extracts

The medicinal plants collected were harvested, dried and ground to

fine powder by a Kenwood multi-mill**.** Phenolic compounds were extracted according to the method described by Sousek et al. (1999). Dry aerial plant material was extracted in Soxhlet apparatus with methanol for 30 min. The solution was used for the analysis of the total phenolic compounds and the estimation of its flavonoid content.

Chemical reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagents were purchased from sigma Aldrich..

Determination of total phenolic content

The total phenolic content was estimated using the Folin-Ciocalteu colorimetric method according to Javanmardi (2003) with minor modifications. Appropriately diluted test sample (0.5 ml) was reacted with 2.5 ml of Folin-Ciocalteu (1/10 N) reagent. The reaction was then neutralized with 2 ml of saturated sodium carbonate (75 g/l) and allowed to stand for 15 min in the dark at 45°C. Later the absorbance of the resulting blue color was measured at 765 nm. Quantification was done on the basis of a standard curve with gallic acid. Results were expressed as gram of gallic acid equivalent (GAE) per 100 g dry weight.

Determination of flavonoid contents

The flavonoid content was estimated using the method of Djerridane et al. (2006). 1.5 ml of ammonium chloride (2%) was added to the same volume of the test sample. The absorbance was measured at 430 nm after a period of incubation at room temperature for 15 min. The control is prepared with the solution of ammonium chloride and the same volume of methanol.

Determination of antioxidant activity

DPPH assay

Antioxidant activity of the samples was determined using the method of Gachtar et al. (2007). 50 µl of the sample was added to 5 ml of DPPH solution (0.004%). The mixture was allowed to stand for 30 min in the dark at room temperature and the absorbance was measured at 517 nm.

H_2O_2 assay

The scavenging effect of H_2O_2 was determined according to the method of Atmani et al. (2009). 1.2 ml of H_2O_2 (40 mM) was added to 2 ml of the sample. After a period of incubation for 10 min at room temperature, the absorbance was measured at 230 nm.

HPLC analysis

HPLC analysis was carried out on a Perkin Elmer series 200 system with C18 (discovery Supelco ODS), 5 µm nucleosil 250 x 16

RESULTS AND DISCUSSION

Phenolic compounds and flavonoid contents

The evaluation of phenolic compounds and flavonoids show that *O. glandulosum* contains 55.15 and 6.88 mg/g of dry weight respectively. The content of phenolic compounds of *O. glandulosum* is three fold lower than that obtained for the aqueous extract of *Origanum vulgare* of Spain (175 mg/g) (Rodriguez-meizoso et al., 2006) and lower than that obtained for *O. vulgare* of Slovenia (Skerget et al., 2005). However, it is higher than that obtained for methanolic extract of *O. vulgare* of Poland (22.21 mg/g) (Capecka et al., 2005).

However, the content of flavonoids for *O. glandulosum* obtained in this study is largely higher than that obtained for *O. vulgare* of Slovenia (Skerget et al., 2005). These differences can be explained by the method of extraction of active substances used in every study, the difference between plant species and the climatic conditions (Fiamegas et al., 2004).

Antioxidant activity

DPPH assay

The IC50 evaluated for the methanolic extract of *O. gladulosum* is equal to 6730 µg/ml. However, the scavenger effect compared to that obtained for gallic acid (IC50 = 43 µg /ml), is not negligible (Table 1). A high scavenger effect is generally due to high content of phenolic compounds, phenolic acids and flavonoids (Samarth et al., 2008; Kouri et al., 2007, Capecka et al., 2005). The content of flavonoids in this study is very low. This partly explains the low scavenging effect of *O. glandulodum* extract.

Scavenger effect of H_2O_2

The scavenger effect of H_2O_2 by methanolic extract of *O. glandulosum* was estimated at 60.78% for a concentration of 66 µg/ml. The synthetic antioxidants like BHA, BHT and α-tocopherol have scavenging effects of 19, 25 and 23%, respectively (Table 2). These effects are largely lower than that obtained for the extract. This can be explained by the presence of phenolic compounds in the methanolic extract of *O. glandulosum*, which can liberate electrons (Balasundram et al., 2005; Elmastas et al., 2006).

HPLC analysis

Phenolic compounds can be defined as a large series of chemical constituents possessing at least one aromatic ring, bearing hydroxyl and other sub-constituents (Ribereau-Gayon, 1968). RP-HPLC analysis is the most used method for the identification of plant phenolic compounds. Because of the diversity and complexity of natural phenolics in medicinal plants, it is difficult to characterize every compound and elucidate its structure.

However, it is not difficult to identify the major categories

Table 1. Effects of methanolic extract of *O. glandulosum* and positive control gallic acid on the free radical (DPPH) scavenging.

Sample	DPPH, IC50 (mg/ml)
Methanolic extract	6730 ± 5.0
Gallic acid	43 ± 0.5

Table 2. Scavenging effect of H_2O_2 by methanolic extract and positive controls (BHA, BHT and α-tocopherol).

Sample	H_2O_2, IC50 (%)
BHA	19 ± 0.3
BHT	25 ±0.25
α-tocopherol	23 ±0.23

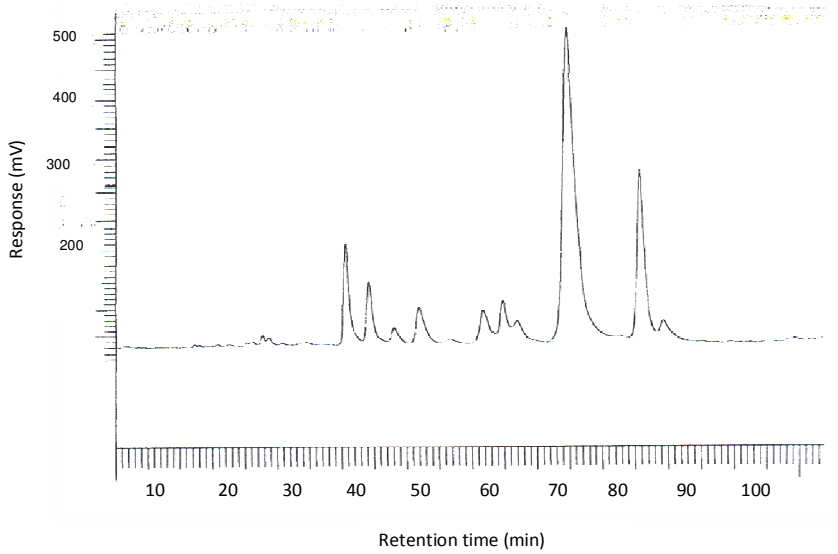

Figure 1. HPLC chromatogram of *O. grandulosum.*

of phenolic compounds.

In the present study, the identification of phenolics of *O. glandulosum* by HPLC was done by the comparison of their retention time with those obtained by Proestos et al. (2006). A typical HPLC chromatogram of *O. glandulosum* is presented in Figure 1. According to the literature, the phenolic compounds of *O. glandulosum* can be successively gallic acid (35 min), vanillic acid (40 min), coumaric acid (50 min), rutin (60 min), ferrulic acid (70 min) and naringenin (85 min).

Conclusion

Free radicals may play an important role in the origin of life and biological evolution. This implies their beneficial effects on the organisms. For example, oxygen radicals exert critical actions such as signal transduction and gene transcription. However, free radicals and other related species could also cause the oxidation of biomolecules which leads to cell injury and death.

In recent decades, the phenolic compounds have been of great interest as they have been in natural products. *O. glandulosum* exhibits a high antioxidant effect compared to that obtained with BHA, BHT and α-tocopherol. Therefore, the results may suggest that *O. glandulosum* possesses compounds with antioxidant properties like ferrulic and gallic acid which could be isolated and then used as antioxidants for the prevention and treatment of free radical related disorders.

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